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APPLICATION NUMBER: 60/345,643

FILING DATE: January 08, 2002

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Docket Number. 791/120

# PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

## INVENTOR(S)/APPLICANT(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
AMIR	ARAV	TEL AVIV, ISRAEL

☐ Additional inventors are being named on page 2 attached hereto

## TITLE OF THE INVENTION (280 characters max)

DIETARY SUPPLEMENT OF OMEGA-9 FATTY ACID (OLIVE OIL) AFFECT THE SPERM MOTILITY AND CONCENTRATION

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## ENCLOSED APPLICATION PARTS (check all that apply)

☒ Specification Number of Pages 5

Applicant is Small Entity

☐ Drawing(s) Number of Sheets

☒ Other (specify)

Assignment

## METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT (check one)

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06-2140

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are

Respectfully submitted,

SIGNATURE

DATE

3 JAN 02

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USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

SEND TO: Box Provisional Application, Assistant Commissioner for Patents, Washington, DC 20231

## **Cryopreservation of large volume of semen permits whole ejaculate and double freezing of bull sperm**

Genetic breeding in the field of dairy cattle includes freezing of sperm collected from young bulls for 4-5 years. In this period the young bull is evaluate by the milk production of his 70-100 daughters. The sperm submitted to artificial insemination (AI) is preserve in liquid nitrogen (LN) in 0.01 cc to 0.05 cc straws.

The bank in the Israeli AI centers preserve 24,000-30,000 straws for each bull.

The expanses for each bull for 4 years are:

1 ton LN (\$1,500) per 24K straws X 4years	\$6,000
60 ejaculate needs 60 days of work (three persons 180X 75)	\$13,500
Food and maintains per bulls (in two years)	\$ 3,200
6 liter freezing extender	\$ 500
24,000 straws	\$ 2,400
<b>Total:</b>	<b>\$ 25,600</b>

Typically only one bull from 14 tested is selected every year for insemination in the dairy herds, which mean that 92% of the straws are discarded without being used.

We suggest here freezing of whole ejaculate in a single test tube of 15ml instead of in 600 straws. After the 5 years of progeny testing, the tubes of the best bulls (8%) can be thawed and refrozen in conventional straws of 0.01 cc to 0.05 cc while the rest of the tubes are discarded. This practice reduces expenses as follows:

400 liter LN (\$600) per 60 test tube per year X 4years	\$2,400
60 ejaculates needs 60 days of work (2 person 120X 75)	\$9,000
Food and maintains per bulls (in two years)	\$3,200
1 liter freezing extender	\$100
60 test tubes	\$6
<b>Total:</b>	<b>\$ 14,706</b>

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The practicality of freezing of whole ejaculates of bull sperm in 15ml test tubes has been compared to conventional freezing of 0.025 cc straws (average of accepted range).

In addition, the effect of the thawing and subsequent re-freezing of the sperm has been evaluated.

For the first freezing of the whole ejaculate in 15ml test tube MTG 1600 (IMT LTD) has been employed with the following parameters:

dT = 5C to -50C;

d=10mm; and

V=1mm/sec.

For the second freezing MTG 700 (IMT LTD) has been employed with the following parameters:

dT= 5 to -50;

d= 0.1mm; and

V=1.5mm/sec.

After storage of 1 to 3 weeks, thawing was done by transferring the straws into water bath of 38C. Viability is express as by post thaw motility of the sperm.

It is clear that large volume freezing in the MTG apparatus is better to the conventional freezing and it permit second freezing with \_ cc straws with overall post thaw survival of 45% which could be use for artificial insemination.

	volume	motility	SMI*	Concentration	Post thaw survival
conventional	4ml	80%	77	$1.2 \times 10^9$	55%
MTG1600 First freezing	4ml	80%	77	$1.2 \times 10^9$	70%
MTG 700 Second freezing					45%

The results are summary of three different bulls.

SMI\*- Semen Motility Index

Figure 1: Effect of omega-3 on sperm motility (column) and conc. (line) vs time

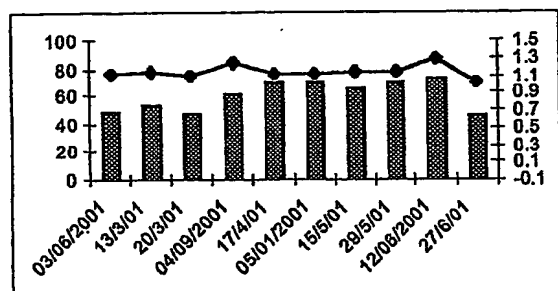


FIGURE 1A

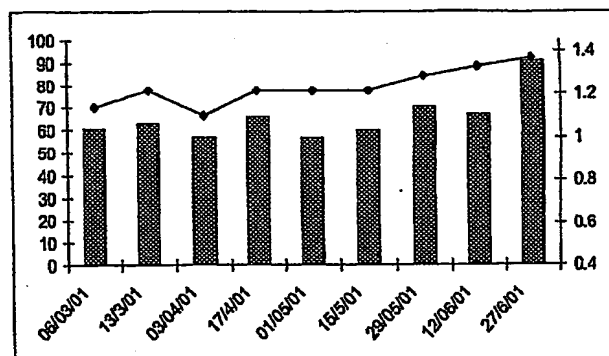


FIGURE 1B

Figure 2: Effect of omega-6 on sperm motility (column) and conc. (line) vs time

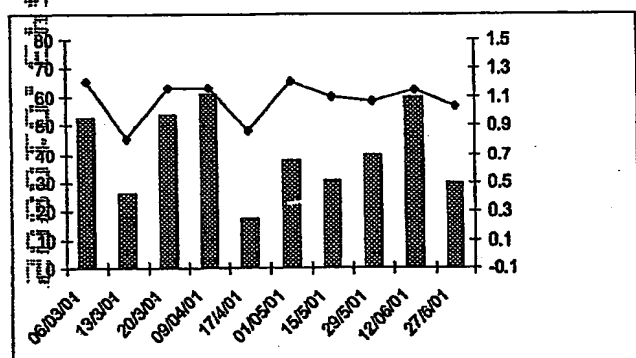


FIGURE 2A

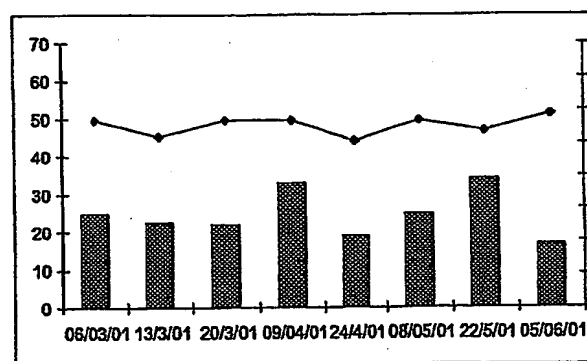


FIGURE 2B

Figure 3: Effect of omega-9 on sperm motility (column) and conc. (line) vs time

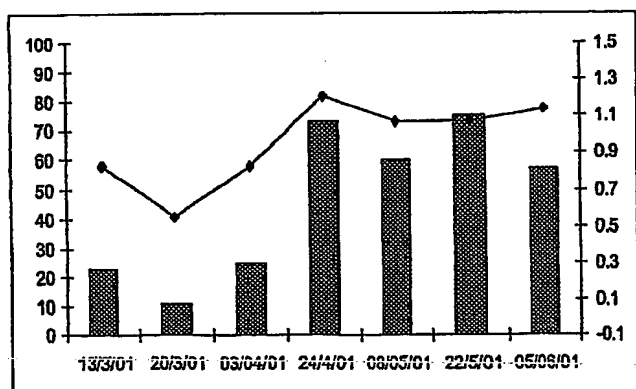


FIGURE 3A

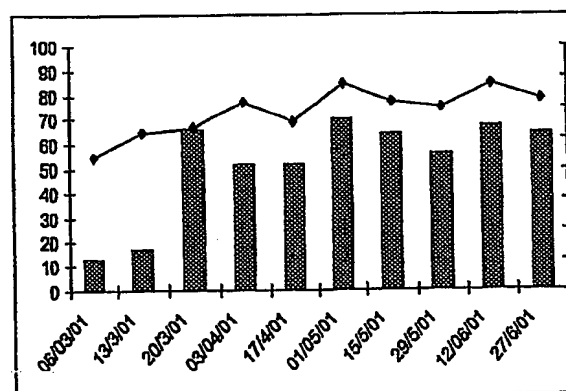


FIGURE 3B

What is claimed:

1. Dietary supplements for improving at least one semen characteristic in ruminant species essentially as described herein.
2. Improved methods for cryopreservation of semen essentially as described herein.

202010-21954203

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Application papers not suitable for publication

SN 60345643

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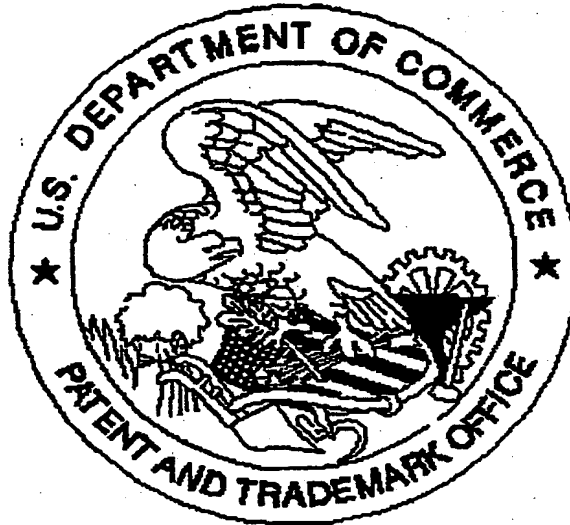
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